Photocatalytic Inactivation of Bacteria by Tio_2-based Compounds under Simulated Sunlight Irradiation

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***Abstract*- $CuCr_2O_4$ nanoparticles have been successfully synthesized via a facile citric acid (CA)-assisted sol-gel method, and further combined with TiO_2 (Degussa P25) by solid phase method. These obtained photocatalysts were characterized by X-ray diffraction (XRD) and UV-vis diffuse reflectance spectrum (UV-vis DRS). Then the photocatalytic inactivation of Escherichia coli, a Gram-negative bacterium, was performed with the photocatalysts in suspension to investigate the photocatalytic bactericidal activities under simulated solar light irradiation. The results show that $CuCr_2O_4/TiO_2$ composite photocatalysts exhibited much better photocatalytic activity than either pure $CuCr_2O_4$ or TiO_2. The effects of $CuCr_2O_4$ to TiO_2 mass ratio, calcination temperature, initial concentrations of *E. coli* and concentrations of catalysts on photocatalytic bactericidal activities over the as-obtained photocatalysts were investigated in detail. The results show that when the concentration of photocatalyst is 0.5 g L^{-1}, the optimal photocatalytic sterilization rate for E. coli (10^5 CFU/mL) over $CuCr_2O_4/TiO_2$ with a 90% content of TiO_2, calcination at 500 °C reached 99.8% within about 40 min, which is 1.62 and 1.33 times more than that of $CuCr_2O_4$ and TiO_2, respectively.**

Keywords*- *Escherichia Coli; $Cucr_2O_4/Tio_2$; Photocatalytic Inactivation; Simulated Sunlight Light

I. INTRODUCTION

Since Matsunaga et al. reported for the first time that TiO_2 photocatalyst could kill bacterial cells in water [1], many research groups have reported the application of semiconductor photo-catalysis to the inactivation of different kind of pathogenic microorganisms, such as bacteria, viruses, algae, fungi or protozoa [2]. The bactericidal effects of TiO_2 photocatalysis, and especially the inactivation of E.coli suspensions, are by far, the most reported studies [3-4]. Several studies have focused on the study on the effects of operational parameters such as light intensity and titanium dioxide concentration [5-6], and the use of solar light[7]. However, TiO_2 has a large band gap(anatase, 3.2 ev; rutile, 3.0 ev), and therefore only UV light (λ<385 nm) can be absorbed, accounting for merely 5% of the sunlight energy. To solve these problems, numerous studies, including impurity doping [8-13], have been performed recently to enhance the photocatalytic efficiency and visible light utilization of TiO_2.

Recently, our group has reported that the metal ions of Cu, Co, Fe doped TiO_2 possesses high photocatalytic activity for H2 production [14-15]. We also found that $CuO/CoFe_2O_4$-TiO_2 possesses better photocatalytic bactericidal effect against E. coli than TiO_2 under simulated solar light irradiation. Because energy gap of CuO (Eg = 1.2eV) is too narrow, the valence band potential may be lower than cell oxidation potential and the narrow-band gap may be occur optical corrosion, which reduce the photocatalytic oxidation ability. The results suggest that the development of better visible light photoatalysts depends on the visible light photoresponse and highly effective interfacial charge-transfer.

In this paper, $CuCr_2O_4$ nanoparticles were synthesized via a facile CA-assisted sol-gel method and $CuCr_2O_4/TiO_2$ composite photocatalysts were prepared by solid phase method, whose photocatalytic activity based on inactivation of the bacteria Escherichia coli was investigated under simulated sunlight irradiation. The effects of several factors of composite photocatalysts on the survival ratio of E. coli were studied.

II. EXPERIMENTAL SECTION

A. Chemicals and Materials

Titania P25 (70% anatase, 30% rutile) was purchased from Degussa Co; Escherichia coli (E.coli DH-5a) was from the Microbiology Laboratory of Hunan Institute of Science and Technology. All other chemicals were of analytical grade. Deionized and doubly distilled water was used throughout this study.

B. Preparation of Photocatalysts

$CuCr_2O_4/TiO_2$ composite photocatalysts were prepared by solid phase method. Briefly, 0.005 mol $Cu(NO_3)_2$ and 0.01 mol $Cr(NO_3)_3$ were dissolved together in 50 ml distilled water to get a mixed solution. The mixed solution was subsequently added into 100 ml 0.3 M citric acid (CA) solution under stirring, and produced a transparent mixed soluble. During this mixing procedure, the temperature was controlled at around 50 °C by using a water bath. Then the temperature was further kept at 80 oC until a transparent and viscous gel was obtained. The as-obtained gel was subsequently transferred into an oven and kept at 130 °C for 3 hours. The as-prepared precursor was then annealed at certain temperature for 1.5 hour with a heating rate of 10 oC /min. $CuCr_2O_4/TiO_2$ heterojunction was synthesized via the same route by adding a certain amount of Degussa P25 TiO_2 in the raw materials preparation when the transparent mixed sol had been obtained.

C. Characterization of Photocatalysts

The crystal phase of the as-prepared photocatalysts were identified by powder X-ray diffraction method (XRD, Bruker D8) using Cu Kα radiation (λ =1.5418Å) at a scan rate of 4°C/min. The UV-vis diffuse reflectance spectrum (UV-vis DRS) of the as-prepared photocatalysts was measured with an UV-vis spectrometer (Shimadzu UV-2500).

D. Photocatalytic Reaction Procedure

Bacteria cell was prepared according to the reference [16]. E. coli, a gram-negative bacterium, was used as model bacteria in this study. They were incubated in Luria-Bertani (LB) nutrient solution at 37 °C for 18 hours with shaking, and then washed by centrifugation at 4000 rpm. The treated cells

were then re-suspended and controlled from 104 to 108 colony-forming units (cfu/mL) with 0.9% saline. All materials used in the experiments were autoclaved at 121 °C for 25 min to ensure sterility. The diluted cell suspension and photocatalyst were added to a 100 mL beaker with a cover. The final photocatalyst concentration was adjusted to 0.6 g/L, and the final bacterial cell concentration was 107cfu/mL. The reaction volume was 30 mL. The reaction mixture was stirred with a magnetic stirrer throughout the experiment. The light source for photocatalysis was a xenon lamp (Model No: DX-150; wavelength, 200-900nm (unfiltered), 150W). Light was passed through without filter and then was focused onto the beaker reactor. With two air exhaust fan for thermal dissipation, the reaction temperature was maintained from room temperature to a maximum 35°C under illumination. A bacterial suspension without photocatalyst was irradiated as a control and the reaction mixture with no light irradiation was used as a dark control. At different intervals during the experiment, certain amount of the reaction solution was taken and diluted with saline and the samples with the appropriate dilution were incubated at 37°C for 18 hours on nutrient agar medium. Then the colonies were counted to determine the number of viable cells. The survival ratio of Escherichia coli was calculated by the ratio of the number of viable colonies and that of viable colonies present initially. All the above experiments were repeated three times and the average values were given.

III. RESULTS AND DISCUSSION

A. Photocatalyst Characterization

Fig. 1 shows XRD patterns of $CuCr_2O_4$/ TiO_2 calcination at different temperature. $CuCr_2O_4/TiO_2$ calcined at 500-700 had better crystallinity. The diffraction peaks of $CuCr_2O_4/TiO_2$ calcined at 300°C were flat, indicating the bad crystallinity. As the increase of calcination temperature, the diffraction peaks of anatase TiO_2 become weaker while that of rutile TiO_2 become stronger, implying the appearance of anatase-to-rutile (A-R) phase transformation. The rutile TiO_2 becomes dominant at a higher calcination temperature. It could clearly be seen that TiO_2 in the composite catalysts presented mainly in the form of rutile, when the calcination temperature is higher than 500°C. In addition to the anatase and rutile phase of TiO_2, the diffraction peaks attributed to $CuCr_2O_4$ were observed. The crystallite size of $CuCr_2O_4$ was 50 nm as calculated with the Debye-Scherrer equation from the line width of the XRD data. The diffraction peaks of $CuCr_2O_4$ become stronger with the increase of calcination temperature, revealing that the crystallinity improves.

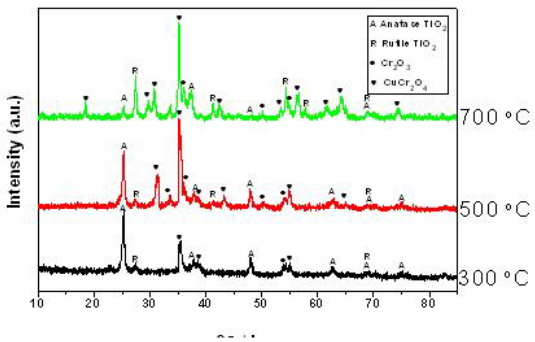

Fig. 1 XRD patterns of the $CuCr_2O_4/TiO_2$ 90% photocatalysts calcined at different temperature

The UV-vis DRS spectra of the photocatalysts is described in Fig. 2. Compared to TiO_2, both $CuCr_2O_4$ and $CuCr_2O_4/TiO_2$ composite exhibit excellent absorption ability, especially $CuCr_2O_4$, which can efficiently absorb the light ranging from UV to visible region

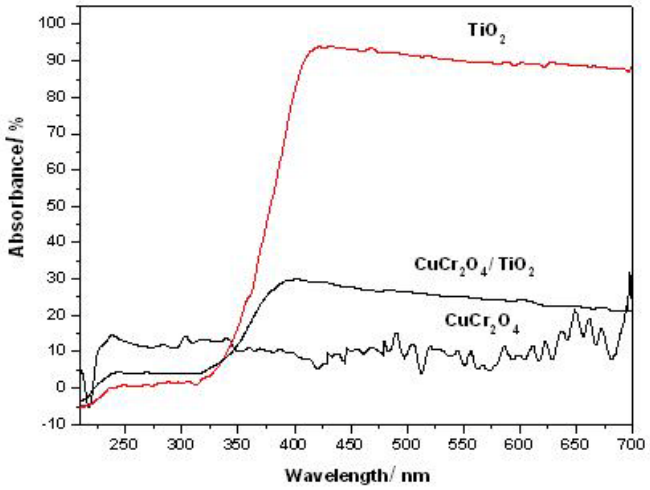

Fig. 2 UV-vis DRS spectra of the different photocatalysts

B. Effect of TiO_2 Content on E. Coli Inactivation

The photocatalytical sterilization result is presented in Fig. 3. The survival ratio of E. coli in the composite photocatalysts is related to the content of TiO_2. The sterilization activity increases linearly with the TiO_2 content until a certain value around 90%, reaching a plateau for higher values. $CuCr_2O_4/TiO_2$ 90% have displayed the maximum killing capacity in the given time and the survival ratio of Escherichia coli decreased to nearly zero in 40 min, whereas other content of TiO_2-based suspension achieved a decrease to nearly zero in 50 or 60 min.

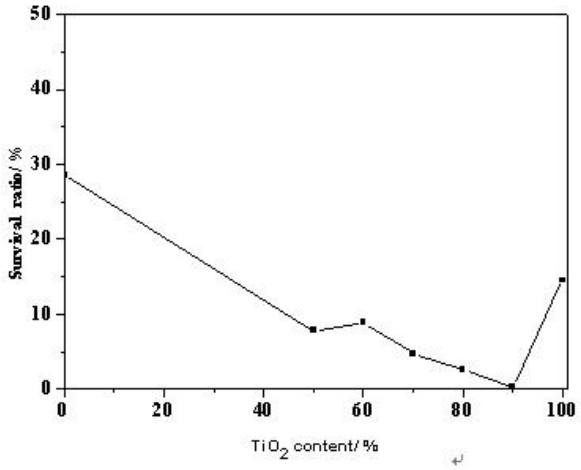

Fig. 3 Effect of different content of TiO_2 on photokilling of E. coli (reaction conditions: calcination temperature: 500°C; irradiation time: 40 min)

These results show that $CuCr_2O_4/TiO_2$ disinfection rate is faster than that of the pure TiO_2, and $CuCr_2O_4/TiO_2$ photocatalyst has promising effects on the disinfection of E. coli, as it enhanced the speed and minimised the time required for the disinfection process. This finding is of direct relevance in applications where treatment time is of importance. Although the amount of $CuCr_2O_4$ with respect to TiO_2 is very small (mass ratio of 1:9), the enhancement of photokilling rate is significant. This effect may arise from the combination of $CuCr_2O_4$ and TiO_2. An appropriate $CuCr_2O_4$ to TiO_2 mass ratio

in the composite can maximize the transfer of photogenerated electrons from $CuCr_2O_4$ to TiO_2, so as to minimise e-h recombination and increase the opportunity for oxidizing surface by positive holes to form increased concentration of hydroxyl radicals (• OH), which in turn have strong oxidative decomposing power[17]. The • OH radicals are even more oxidative thanmO_2- and can react with organic matter such as cells and E. coli to destroy them[18].

C. Effect of Calcination Temperature

Fig. 4 summarizes the survival ratio of E. coli using the $CuCr_2O_4/TiO_2$ 90% as a function of the calcination temperature. The survival ratio of E. coli decreases with the calcination temperature from 200 to 500°C, reaches its minimum at 500°C, and thereafter increases. Good crystallinity is very important to the activity of catalyst [19]. As is shown in the XRD results (Fig. 1), the $CuCr_2O_4$ shows poor crystallinity at lower calcination temperature, and the crystallinity improves as the calcination temperature increases, resulting in lower survival ratio of E. coli. The survival ratio of E. coli is only 6.8% within 30 min under the xenon lamp irradiation when the calcination temperature is 500°C. Although a higher calcination temperature can improve the crystallinity, it can also decrease the surface area of nanoparticles and cause the TiO_2 change from anatase to rutile [20]. As a result of small surface, it can absorb fewer photons and provide a longer transfer path, leading to more recombination of electron and hole, on the other hand, at higher temperature, catalyst transforms to the rutile phase that its catalytic activity is very poor[21]. Therefore, the photocatalytic inactivated rate of E. coli decreases at higher calcination temperature.

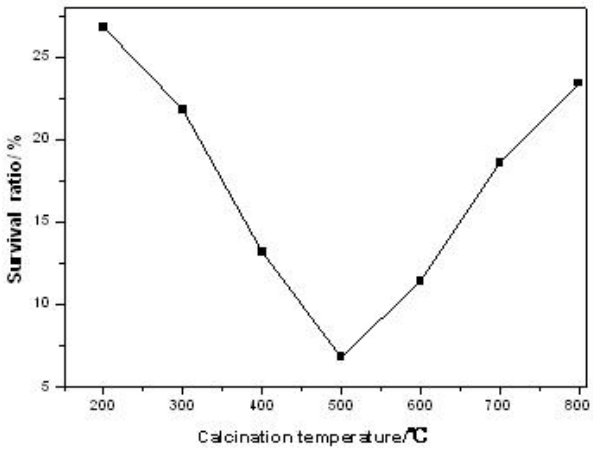

Fig. 4 Effect of the calcination temperature on the survival ratio of Escherichia coli over the as-obtained CuCr2O4/TiO2 90%

D. Effect of Different Initial Concentrations of E. Coli

The effects of different initial concentrations of E. coli (104-106 CFU/mL) on photacatalytic activity are shown in Fig. 5. From the figure, it is clear that with the increase of initial concentration of E. coli, the complete sterilization time using CuCr2O4/TiO2 90% is correspondingly increased. 104 CFU/mL and 105 CFU/mL of E. coli are completely inactivated within 40 min, and under the same conditions it takes 60 min to completely kill 106 CFU/mL of E. coli. Some reports found that the rate of bacterial inactivation in prophase was faster than that of anaphase where it needed a long time to kill the remaining cells, and the result met the first-grade kinetics rule [22].

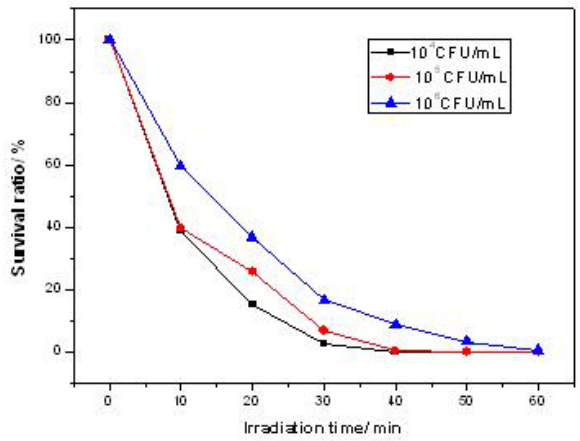

Fig. 5 Effect of different initial concentrations of E. coli on the survival ratio of Escherichia coli over the as-obtained $CuCr_2O_4/TiO_2$ 90% calcined at 500°C

E. Effect of the Photocatalyst Concentration

The photocatalytic bactericidal activities of $CuCr_2O_4/TiO_2$ 90% with different concentrations are shown in Fig. 6. The activity increases with the increase of the photocatalyst concentrations. However, the photocatalytic bactericidal rate reaches its maximum at the photocatalyst concentration of 0.5 g L−1. And Escherichia coli were almost completely inactivated within about 40 min when the initial concentration of E. coli is 105 CFU/mL. It is well known that the active centre number on catalyst particle surfaces and the penetration ability of incident light in reactor are extremely important for the photocatalytic activity [23]. Generally, larger catalyst concentration provides more catalytically active centres for the absorption of photons, and then more electrons and holes are generated. However, the excess photocatalyst may act as an optical filter and impede the further penetration of incident light into the suspension. We observed that the photocatalytic bactericidal activity increases with increasing the $CuCr_2O_4/TiO_2$ 90% concentrations when the concentration is lower than 0.5 g L−1, whereas it decreases with further increasing photocatalyst. In addition, the activated particles can be deactivated by the collision with the inactivated particles which act as electron-hole trappers, resulting in charge loss.

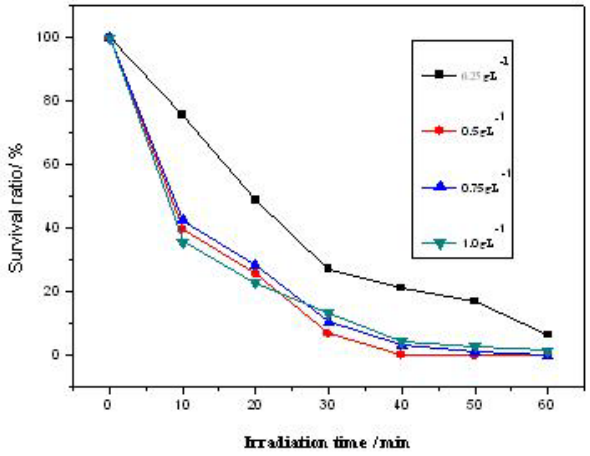

Fig. 6 Effect of the photocatalyst concentration on the survival ratio of Escherichia coli over the as-obtained $CuCr_2O_4/TiO_2$ 90% calcined at 500°C

F. Effect of Different Experimental Conditions

Fig. 7 illustrates the survival ratio of E. coli under

simulated sunlight or dark condition. The sterilization activity of $CuCr_2O_4/TiO_2$ is superior to TiO_2 or $CuCr_2O_4$ under simulated solar light irradiation. The optimal photocatalytic sterilization amount of $CuCr_2O_4/TiO_2$ is 1.62 and 1.33 times more than that of $CuCr_2O_4$ and TiO_2, respectively, which owing to the appropriate combination of $CuCr_2O_4$ and TiO_2 can help to the transfer of photogenerated electrons from $CuCr_2O_4$ to TiO_2, improving the separation of photogenerated e-h pairs, as discussed above in section 3.2. It was noted that the Pd additive can also promote visible-light absorption in nitrogen-doped TiO_2 [24]. The visible light absorption is essential to the charge production on the semiconductor photocatalyst [25]. Such dual roles of $CuCr_2O_4$ significantly enhanced the photocatalytic activity of $CuCr_2O_4/TiO_2$ under visible-light illumination.

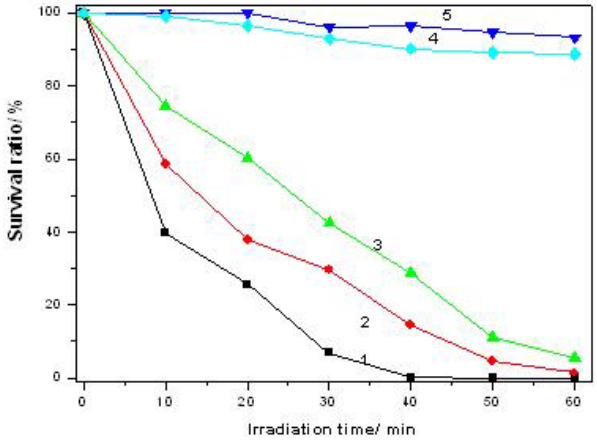

Fig. 7 Comparison of survival ratio of E.coli under different experimental conditions (reaction conditions: $mCuCr_2O_4$: $mTiO_2$=1: 9; irradiation time: 60 min): 1. E. coli + $CuCr_2O_4/TiO_2$+light; 2. E. coli +TiO_2+ light; 3. E. coli + $CuCr_2O_4$+ light; 4. Light only without catalyst; 5. E.coli + $CuCr_2O_4/TiO_2$ + dark

The result of this study also showed that there is no significance change in microbial count of the $CuCr_2O_4/TiO_2$ photocatalyst placed in the dark (Fig. 7). The survival ratio of E. coli still reaches 93.21% after 60 min irradiation. Minor variations in the bacterial count are due to experimental errors and variations arising during the sampling procedure where "true" darkness could not be ascertained. Therefore, the $CuCr_2O_4/TiO_2$ itself is nontoxic to E. coli.

Irradiation without catalyst doesn't exhibit a good effect of sterilization. After 60 min, the survival ratio of E. coli still reaches 88.72%. Comparing with dark condition, the antibacterial property under simulated sunlight condition with $CuCr_2O_4/TiO_2$ 90% has a great improvement, and the survival ratio of E. coli is only 6.8% within 30 min under the xenon lamp irradiation. Therefore, both light and catalysts play a significant role on photocatalytic inactivation of E. coli.

IV. CONCLUSIONS

$CuCr_2O_4/TiO_2$ composite photocatalysts were successfully synthesized via the facile citric acid (CA)-assisted sol-gel method and solid phase method. The as-obtained photocatalysts exhibits higher photocatalytic sterilization activity compared with pure $CuCr_2O_4$ or TiO_2 for killing Escheri chia coli under the xenon lamp of 150W irradiation. When the concentration of photocatalyst is 0.5 g L^{-1}, the optimal photocatalytic sterilization rate for E. coli (105 CFU/mL) over $CuCr_2O_4/TiO_2$ 90%, calcination at 500C can be achieved, 99.8% Escherichia coli can be sterilized within 40 min under simulated sunlight irradiation.

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REFERENCES

[1] T. Matsunaga, R.Tomoda, T. Nakajima, H. Wake, FEMS Microbiol. Lett. 29 (1985) 211-214.

[2] C. McCullagh, J. M. C. Robertson, D. W. Bahnemann, P. K. J. Robertson, Res. Chem. Intermed. 33 (2007) 359-375.

[3] P. S. M. Dunlop, J. A. Byrne, N. Manga, B. R. Eggins, J. Photochem.Photobiol. A: Chem. 148 (2002) 355-363.

[4] H. M. Coleman, C. P. Marquis, J. A. Scott, C. C. Chin, R. Amal, Chem. Eng. J. 113 (2005) 55-63.

[5] Y. Horie, D. A. David, M. Taya, S. Tone, Ind. Eng. Chem. Res. 35 (1996) 3920-3926.

[6] A. K. Benabbou, Z. Derriche, C. Felix, P. Lejeune, C. Guillard, Appl. Catal. B-Environ. 76 (2007) 257-263.

[7] P. Ferna ′ndez, J. Blanco, C. Sichel, S. Malato, Catal. Today. 101 (2005) 345-352.

[8] S. Takashi, T. Masahito, Biochem. Eng. J. 30 (2006) 199-204.

[9] L. Li, M. Lei, T. Sakae, Mater. Sci. Eng., B. 148 (2008) 183-186.

[10] S. Funda, A. Meltem, K. Nadir, J. Hazard. Mater. 162 (2009) 1309-1316.

[11] H. Irie, Y. Watanabe, K. Hashimoto, Chem. Lett. 32 (2003) 772-773.

[12] D. M. Chen, D. Yang, Q. Wang, Z. Y. Jiang, Ind. Eng. Chem. Res. 45(2006) 4110-4116.

[13] V. Subramanian, E. E. Wolf, P. Kamat, J. Am. Chem. Soc. 126(2004) 4943-4950.

[14] Yang, H. H.; Yan, J. H.; Lu, Z.G. *J.* Alloys. Compd. 476(2009) 715-719.

[15] Yan, J. H.; Yang, H. H.; Tang, Y. G. *Renew Energy*. 34(2009) 2399-2405.

[16] Y. Q. Lan, C. Hu, X. X. Hu, Appl. Catal. B-Environ. 73(2007) 354-360.

[17] A. Vohra, D.Y. Goswami, D. A. Deshpande, S. S. Block, J Ind Microbiol Biotechnol. 32 (2005) 364-370.

[18] R. Vasant Kumar, R. Ghulam, Ionics. 15 (2009) 579-587.

[19] S. T. Valdes, G. Marban, A. B. Fuertes, Catal. Today. 116 (2006)354-360.

[20] T. Tsumura, N. Kojitani, I. Izumi, N. Iwashita, M. Toyoda, M. Inagaki, J. Mater. Chem. 12 (2002) 1391-1396.

[21] M. Hamadanian, A. Reisi-Vanani, A. Majedi, Appl. Surf. Sci. 256(2010) 1837-1844.

[22] Rincon, A. G.; Pulgarin, C. *Appl. Cata. B: Environmental.* 2004, 44, 263.

[23] S. Ullah, A. H. Dogar, N. Mehmood N, Ion-induced secondary electron emission from MgO and Y_2O_3 thin films, Vacuum 84 (2010) 509-513.

[24] Q. Li, R. Xie, E. A. Mintz, J. K. Shang, J. Am. Ceram. Soc. 90 (2007) 3863-3868.

[25] P.G. Wu, R. C. Xie , J. A. Imlay, J. K. Shang, Appl. Catal. B-Environ. 88 (2009) 576-581.